Bulletin 222 April, 1925

STUDIES WITH ANTHRACNOSE INFECTION IN COTTON SEED

C. A. LUDWIG,
Associate Botanist and Plant Pathologist.

A cotton plant badly diseased with anthracnose

SOUTH CAROINA AGRICULTURAL EXPERIMENT STATION
OF CLEMSON COLLEGE
H. W. BARRE, Director
Clemson College, S. C.

Studies With Anthracnose Infection in Cotton Seed

C. A. LUDWIG,
Associate Botanist and Plant Pathologist.

INTRODUCTION

Anthracnose is one of the major pests of cotton. Next to the boll weevil this disease and wilt are probably the most destructive enemies of the cotton crop in this country. In 1909 Barre (5) estimated the annual loss from the disease at 1 percent in South Carolina. The next year the same author (6) estimated that a very conservative figure would be $400,000 to $500,000. The loss that year (8) was estimated at more than a million dollars. DeLoach (23) in 1909, claimed an annual loss of 17 percent in Georgia, which on the basis of forty dollars per bale amounted to $14,756,000. Estimates compiled by the Plant Disease Survey of the United States Department of Agriculture (34) for the entire cotton belt for the years 1917 to 1921 inclusive, range from 1.6 to 3.9 percent, an average 2.8 percent, or about 358,000 bales. Additional estimates could be cited but they are of about the same order of magnitude as these and would add little to a statement of the importance of the disease. It is not so important, however, as it was a decade or so ago, since control measures are now available by which it can effectually be held in check.

The anthracnose fungus in found throughout the American cotton belt, and the same or a very similar one has been reported in British Guiana (21), the West Indies (28, 29, 30, 36), and British West Africa (33, p. 20). It is stated that in this last named region "native cottons are susceptible to anthracnose (Colletotrichum Gossipii). American cottons and hybrids are very little subject to this disease." This condition seems rather odd in view of the damage which the disease does in this country. It is reported by Balls as common in Egypt, although apparently it is not a serious pest (4, p. 88); and Butler reports it as rare in India (22). The same author states that it is found in the West Indies, South and West Africa, Bulgaria, and Trans-Caucasia, but does

not cite authorities for his statement. Welles (39) has recently recorded it as newly discovered in the Philippines, while Birmingham and Hamilton (21) have reported it in New South Wales, Australia. In short, the disease seems to have been found in about every large region where cotton is grown to any extent except South America and China; and the presumption is that it occurs in these regions also. This is especially likely, since, in China at least, importations of North American varieties have been made.

HISTORICAL STATEMENT

Cotton anthracnose has been known to students of plant pathology since 1891, at which time Miss Southworth (37,38) and Atkinson (1, 2) studied and described it. Miss Southworth named the fungus **Colletotrichum gossypii.** Later Shear and Wood (35) reported that they had obtained an ascogenous stage in culture. Still later Edgerton (25) reported finding perithecia of the fungus on cotton bolls in the field and transferred it to the genus Glomerella.

From the first it was recognized that the fungus is carried over from one season to the next and disseminated throughout the country by means of the seed. Atkinson (2) thought that cotyledon infection must have its source in spores lodged on the lint, although later (16) recognizing the possibility that tissue invasion might be involved. He raised infected seedlings in the greenhouse at Cornell University, Ithaca, New York, merely by planting infected seed (3), thus giving very good proof of the possibility of disseminating the disease by this means.

Following Atkinson's work on the role of seed infection in the dissemination of the disease little was done along this line for over a decade, when DeLoach (23) and Barre (5) demonstrated independently of each other that internal infection of the seeds is common. Both considered that slight infections, not sufficient to kill the seeds, would serve to transmit the disease; but Barre alone summitted data definitely in support of the idea. He showed that surface steri-

lization of the seed will not eliminate anthracnose from the seedlings and crop resulting.

Barre followed this work up (7, 10, 11, 12) with a series of observations and experiments which showed clearly that diseased seed is an important agency which carries the disease from season to season and from locality to locality, that seed and other infected debris in the field will harbor the fungus in living condition long enough to infect a succeeding crop, and that the fungus in infected seed stored in the laboratory dies out in about three years time. He noted, however, (16) that seed claimed by farmers to be three years old was not always free of infection.

Tests of a number of different methods of control have been reported. As was naturally to be expected, some of the earliest of these, based on the fact that the seed carries the disease, were aimed at seed sterilization. Thus Atkinson (2), thinking that cotyledon infection was from lint-borne spores, was of the opinion that scalding the seed might prevent the disease if the seed were planted in soil not in cotton the preceding season. His results gave some encouragement to this view, since no diseased seedlings were produced by scalded seed planted in sterilized soil in the Cornell University greenhouse.

Barre (5), however, seems to have been the first to experiment on any considerable scale with seed treatments. He used various strengths of copper sulphate, mercuric chloride, and formaldehyde, but with disappointing results, as none of the treatments seemed to reduce infection appreciably.

Duggar and Cauthen (24) tried the following methods of treatment on a diseased lot of seed: Hot water, 170 degrees F., 10 minutes; formalin, 4 percent, 30 minutes; no treatment; seed coat charred with pure sulphuric acid; copper sulphate, 10 percent, 1 hour; fumigated with carbon disulphide; formalin, 5 percent, 30 minutes; no treatment; and hot water, 150 degrees F., 22 minutes. The effectiveness of the treatments

was checked by determining the percentage of infected bolls in the crop grown from the different lots of seed. This method has the objection that it might not show the true value of a successful treatment unless the possibility of outside infection during the growing season were carefully eliminated. The results as secured, however, showed some reductions of disease for all treatments, the greatest being for the hot water treatments. This is about what would be expected, as each treatment should kill all or at least part of the spores on the outside of the seed, while the hot water treatment alone could be expected to dispose of any of the internal infection.

Edgerton (26) reported a few tests with formaldehyde in 1912. The treatment reduced the number of diseased seedlings about half but still left a heavy infection.

Barre (13) in 1913, noted the failure of delinting the seed with strong sulphuric acid to prevent the transmission of the disease.

Experiments designed to develop a method of destroying the fungus within diseased seeds was reported by Barre (14, 15) in 1914 and 1915. At first a hot water treatment gave promising results. However, it later proved unsatisfactory, partly because the fungus in an occasional infected seed seemed to live through the treatment and partly because different samples of cotton seed showed different degrees of resistance to the heat. Results recently reported by Lipscomb and Corley (31, 32) are suggestive as indicating that possibly the variation in the reaction of cotton seed to heat is due to variation in moisture content rather than to varietal or other constitutional differences. These authors found that cotton seed with a "normal" amount of moisture will be killed if heated in water to 70 to 80 degrees C., but if thoroughly dried at a low temperature can be heated for 10 to 15 minutes at 100 degrees in a dry atmosphere without affecting their vitality. In a vacuum or in an inert gas, free of oxygen, the heating can be prolonged, or conducted at a higher temperature than 100 degrees, as much as 26 hours at 100 degrees C., or 10 hours at 110 degrees C. One hour at 120 degrees decreased

germination only slightly. They claim that the longer periods of heating killed all internal anthracnose infection, but there are so few trials reported and the details given are so meager as to make it impossible to evaluate this part of their work with assurance.

It has been known for some time that the fungus in the seed dies out in storage. Barre (5) in 1909 reported experiments which showed that, in some cases at least, more than fourteen to fifteen months is required for that result. Later (11) he found that the fungus sometimes lives as long as to the end of the third year. An experiment by Edgerton (26) in Louisiana, shows, however, that in some cases, at least, the fungus perishes in about thirteen months in that state.

The fact just mentioned, that the fungus dies out with long storage, has suggested that the result is brought about by the drying of the seed; and in 1919 Barre (17), reporting work of Lipscomb and Wilson in this laboratory, stated that the anthracnose fungus in the seeds could apparently be killed by drying them in a vacuum. Later results have not fulfilled the promise which the early tests gave; and his latest reports indicate that the death of the fungus cannot be due to simple drying, since drying by ordinary methods does not reduce infection to any marked extent (18).

But, while no quick method of destroying the infection in cotton seed has been discovered, practical means of control for the disease have been developed. These depend on the observance of sanitary measures based on a number of facts concerning the fungus. Barre's work (7, 9, 10, 13) demonstrated that clean seed planted in clean soil will produce a clean crop and (9, 10, 13) that, while the disease will live from one season to the next on infected trash it will not live longer, and will not live that long if the trash is plowed into the soil. Further work by the same investigator (10, 16) showed that, while many slightly diseased or apparently healthy bolls yield infected seed, it is possible to select clean seed from a diseased crop provided a considerable percentage of the plants is clean.

The plan recommended is to get clean seed and start anew if the infestation is very great. If it is not too great some one familiar with the disease should go through the field ahead of the pickers at picking time and mark in some manner all plants containing any diseased bolls. All the cotton on these plants and the ones near them is then picked and discarded for seed purposes. The rest of the cotton is then picked into clean bags, ginned in a gin from which all seed from previous ginnings has been carefully removed, the seed delinted with strong sulphuric acid, and used for planting. A few years of this sort of selection carefully carried out will produce a crop free from the disease provided plantings are made on clean soil.

Of course any other method of selection by which only clean seed are planted will accomplish the purpose. In particular, the plant-to-row method of selection which has proved so valuable in breeding work will give first class results. This really gives an opportunity to get more rapid results than the mass selection method mentioned above, as it is usually easier to find single disease free plants not close to any diseased ones than it is to harvest a large amount of clean seed from a field with considerable disease in it.

It will be an advantage, as indicated elsewhere, to delint all seed for planting with strong sulphuric acid. This is especially true if there is reason to suspect that the gin had previously been used on diseased cotton. The delinting will kill all or nearly all the spores which cling to the outside of the seeds. Careful distinction should be made in this connection between delinting with sulphuric acid and delinting with a gin set to run close. Most of the fuzz can be removed by the latter method but it does not kill adhering spores.

Another way of getting clean seed is to store the seed in a dry place for at least two years before planting.

Having secured clean seed, it is next necessary to plant them in clean soil. This, as implied above, requires rotation of crops or plowing under of stalks in the fall unless the previous crop was free from the disease.

Attention to these precautions has made the anthracnose problem a much less serious one in South Carolina than it formerly was. In fact, we have had difficulty at times in the last few years in securing diseased seeds for our experiments. North Carolina has adopted essentially these measures and has developed a cotton seed certification program (27) in which freedom from disease is one of the requirements for certification.

EXPERIMENTAL

Aim

When it became apparent that drying cotton seed in a vacuum would not eliminate anthracnose infection it was decided to carry through an experiment with the idea of getting as much light as possible on the cause of the mortality of the fungus in stored cotton seed and of determining as accurately as possible the best conditions for the storage of infected seed. The results of this work form the subject matter of most of the discusssion which follows, although certain other experiments are also described. The following features have been studied and are here presented: The "normal" mortality curve for anthracnose infection in cotton seed in storage, the effect of delinting and sterilizing, of heating air-dry seed, of drying the seed, of storage under laboratory conditions (arbitrarily taken as the standard), storage over a radiator, storage in a 30 degree incubator, storage outside under a roof, storage in a moist chamber, storage in dessicators, both in the open laboratory and in a 30 degree incubator, storage alternating between a moist chamber and a dessicator, of combinations of different seed treatments and storage methods, and of sunning.

General Methods

In all this work heavily infected cotton seed were used. The degree of infection was determined in all cases, both before and after treatment, by germinating the seed in sterile test tubes and examining the plantlets microscopically for the fungus. During the first part of the investigation, or until April, 1921, the seed were germinated on moistened blotter

paper in the bottoms of the test tubes by the method developed by Barre and Aull (19). At that time a series of tests was run in which 50 percent of the seed were germinated in

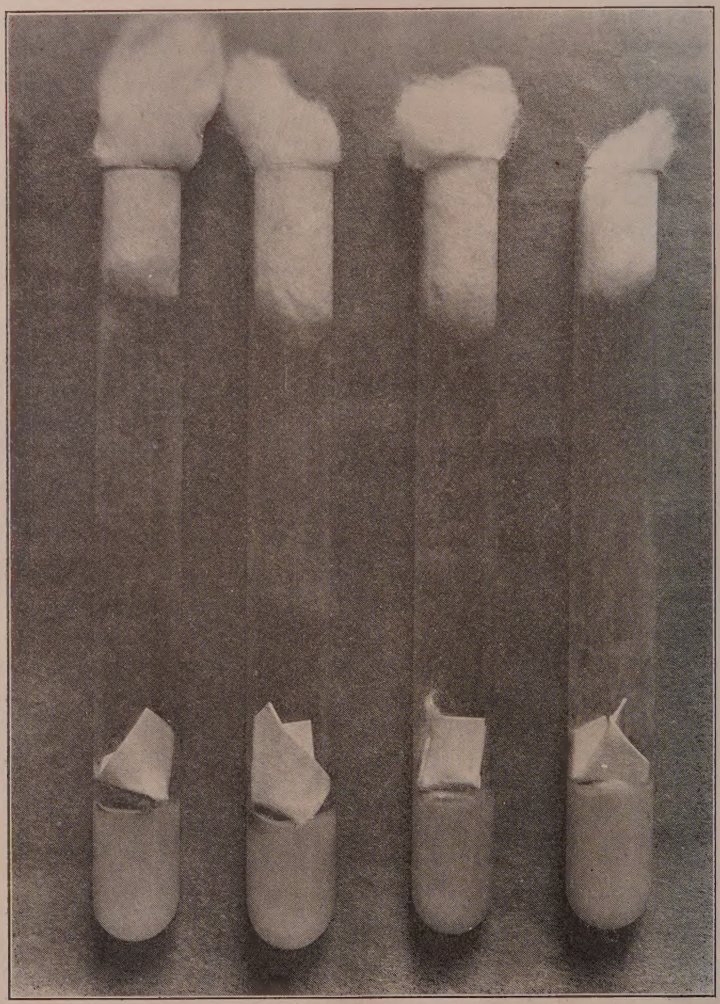

Fig. 1.—Method of planting cotton seeds on agar for determination of anthracnose infection.

the usual way and the other half on 3 percent non-nutrient agar instead of moistened paper. As no difference was apparent in the results and as the agar is more easily prepared and cleaned from the tubes after using, it alone was used in all subsequent tests. In all cases each seed was covered with

Fig. 2. Cotton seedlings in test tubes. The one at the right is diseased at the base. The rest are healthy.

a small piece of sterilized blotter paper to hold it firmly against the substratum. This is necessary in order to insure germination and normal development. Fig. 1 shows four seeds immediately after planting and Fig. 2 shows four seedlings produced in this way. The right hand seedling is diseased at the base with anthracnose. The others are healthy.

The seed were germinated for the most part, at a temperature of approximately 30 degrees C., a large incubator with electric heating and control devices being used for the purpose. In summer no extra heat was used, as the room temperature often approximated 30 degrees, and rapid germination of the seed and development of the seedlings occurred without additional heat. No attempt was made to control the temperature closely but merely to keep it high enough for rapid germination and development.

At first the samples consisted of fifty seeds. It soon became apparent, however, that the probable error of results secured from so small a sample is rather great; and the size of sample was increased to seventy-five seeds. Determinations based on any considerable number of seeds more or less than seventy-five are so marked in the tables recording the results. Accidental variations of only a few seeds are not noted.

Records are given in the tables of percent of germination, percent of seedlings infected with anthracnose, and percent of seedlings without anthracnose. In most cases the sum of the last two should equal the first, as all are calculated on the basis of the number of seeds put to germinate. Occasionally, however, owing to the loss of one or more tubes after germination of the seeds and before final examination of the plantlets, this does not hold true. In drawing the curves the percentages of anthracnose for the different dates were plotted and smooth curves drawn in freehand by inspection. No claim is made for mathematical accuracy of the curves but merely that they indicate with fair degree of approximation the course of the changes represented. Smooth curves have been used rather than broken-line curves because with the

large number of data presented per figure the latter would be confusing. For the same reason a few curves, but not the plotted points, are omitted in one or two of the figures.

Three lots of seed were used. The work in 1920 was done with seed of the Cook variety. This lot of seed was secured from diseased bolls selected from the main crop on the college farm and, as the following records show, was heavily infested with the fungus (a).

The bulk of the work was done with a heavily infected sample of Toole seed, secured in the early winter of 1920 through the courtesy of Mr. H. K. Sanders, County Agent of Chester County, South Carolina. In spite of the fact that it was a gin run sample, and not selected in the field for infection, the infection was very high.

The storage work in alternating very dry and very moist conditions was done with a heavily infected sample of Webber 82 seed which was raised in one of the experimental plots of the South Carolina Experiment Station in 1922 by planting infected seed.

Each of these lots of seed will be indicated in the following pages by the approximate variety name.

The "Normal" Mortality Curve of the Fungus

The mortality curve of the fungus which accompanies storage of the seed under usual laboratory conditions is arbitrarily taken as "normal" for the purposes of this study. As a matter of fact, farm storage conditions and the course of the death curve under those conditions are probably more properly entitled to this term. For our purpose, however, one is as good as the other; and there are more data at hand for the former than for the latter.

Tables 1 to 3 and Fig. 3 show the course which this curve took for each of the three lots of cotton seed concerned in this study. It will be noted that the amount of anthracnose remains high for several months and then decreases rapidly for a few months along about the time the seed is a year old.

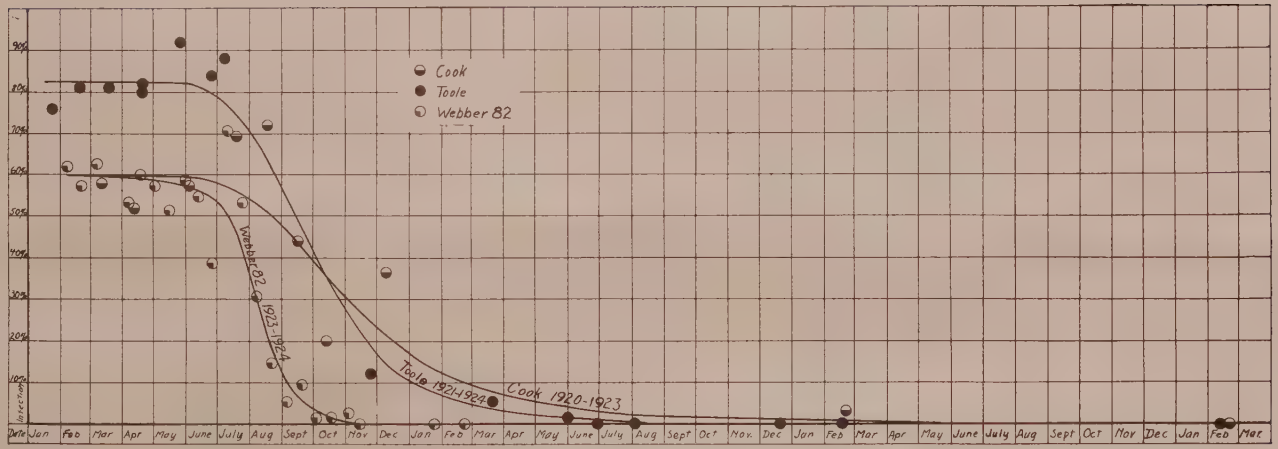

Fig. 3.—Curves showing the "normal" rate of decrease of the anthracnose fungus in the three samples of cotton seed used in this study.

By the second planting season the amount of infection is very small and by the third the amount remaining may be neglected for all practical purposes.

It was impossible with either lot of seed to get determinations immediately after ginning. However, as the slope of the curves does not appear to change greatly till much later in the history of the seed it seems fair to assume that little change had taken place when the first determinations were made.

Table I.—Percent of Anthracnose Infection in Cook Cotton Seed at Various Dates

Date	No. Seeds	% Germination	% Seedlings with Anthracnose	% Seedlings without Anthracnose
1920				
March 12	50	72.0	58.0	14.0
April 12	50	76.0	52.0	24.0
June 4	75	74.7	57.3	17.3
July 19	75	73.3	69.3	4.0
August 18	75	77.3	72.0	5.3
September 16	75	76.0	44.0	32.0
October 13	75	73.3	20.0	53.3
December 9	74	85.1	36.5	48.6
1922				
February 21	100	79.0	3.0	76.0
1923				
February 21	150	83.3	0.0	83.3

(a) This lot of seed was gathered by Mr. J. L. Seal and Mr. M. L. McHugh, thanks for which is hereby extended to them. Thanks are also extended to the following for other services in connection with these studies: Mr. H. K. Sanders, County Agent of Chester County, South Carolina, for the infected Toole cotton seed used for the main experiment; Mr. M. L. McHugh, Mr. W. C. Cook, Mr. L. E. Tisdale, Mr. F. G. Tarbox, Mr. Claude Schilletter, Mrs. W. B. Aull for laboratory work and microscopic examinations; Professor William Lippincott, of the Chemistry Division of the South Carolina Experiment Station, for three moisture determinations; and to Professor H. W. Barre, Director and Botanist of the South Carolina Experiment Station, for counsel and advice.

Table 2.—Percent of Anthracnose Infection in Toole Cotton Seed at Various Dates

Date	1921									1922					1923	1924
	1-24	2-19	3-19	4-21	4-19-22	5-27	6-25	7-8	11-25	3-21	5-31	6-29	8-3	12-20	2-17	2-12
% Germination	*77.4	‡85.1	90.6	90.4	82.7	93.3	†89.3	93.3	93.3	93.3	94.7	94.7	85.3	94.5	93.3	97.3
% Seedlings with Anthracnos	76.4	81.2	81.2	82.2	80.0	92.0	84.0	88.0	12.0	5.3	1.3	0.0	0.0	0.0	0.0	0.0
% Seedlings without anthracnose	1.0	4.0	10.3	8.2	2.7	1.3	5.3	5.3	81.3	88.0	93.3	94.7	85.3	94.5	93.3	97.3

* 198 seeds.
‡ 101 seeds.
† From original lot of seed stored on a shelf in a herbarium case.

Table 3.—Percent of Anthracnose Infection in Webber 82 Cotton Seed at Various Dates

Date	1923																		1924	
	2-7	2-21	3-7	4-7	4-18	5-2	5-16	5-31	6-13	6-27	7-11	7-25	8-8	8-22	9-5	9-20	10-3	10-18	11-3	11-14	1-24	2-22
% Germination	86.5	85.3	97.3	96.0	94.7	90.7	94.6	96.0	93.3	96.0	96.0	97.3	94.7	98.7	98.7	98.7	94.7	*95.7	94.7	94.7	98.7	97.3
% Seedlings with Anthracnose	62.2	57.3	62.7	53.3	60.0	57.3	51.4	58.7	54.7	38.7	70.7	53.3	30.7	14.7	5.3	9.3	1.3	1.4	2.7	0.0	0.0	0.0
% Seedlings without Anthracnose	24.3	28.0	34.7	42.7	34.7	33.3	43.2	37.3	38.7	57.3	25.3	44.0	64.0	84.0	93.3	89.3	93.3	94.3	92.0	94.7	98.7	97.3

* 70 seeds only.

The Main Series of Experiments

In the main experiments the Toole seed were used. These seed were received at Clemson College in January, 1921, and were stored in the laboratory until the lots were treated and put in the various types of storage. They had a moisture content on April 16 of 6.47 percent.

The seed were divided into six lots and treated as follows: A, check, no treatment; B, dried for a limited time in a commercial (Ellis) drier; C, dried almost to constant weight in the commercial drier; D, heated two hours at approximately 65 degrees C. in a hot air oven; E, delinted and sterilized; F, delinted, sterilized, and dried in the commercial drier.

The seed in each of these lots, except C, were further divided into seven lots and were stored as follows: (1) In a paper bag in the laboratory; (2) in a paper bag in a 30 degree incubator; (3) in a paper bag over a radiator; (4) in a paper bag outside a window opening on a north portico; (5) in a paper bag in a case containing an open dish of water; (6) over calcuim chloride in a dessicator in the laboratory; and (7) over calcuim chloride in a dessicator in the 30 degree incubator. Lot C was not large enough to be divided into more than four portions, and the methods of storage adopted for it were 1, 4, 5, and 6, as mentioned above. There were thus, in all, thirty-nine combinations of seed treatment and storage under examination.

It was planned to make an anthracnose determination on each lot of seed as often as once a month but it was not possible to keep up with this schedule very closely. In fact, owing to the resignation of the laboratory assistant, there is a break of about four months during the autumn of 1921 in the record for each lot. This break is exceedingly unfortunate because it occurs at the period when the greatest decrease in the amount of infection was taking place.

The different portions of Lot A were put in storage under their respective conditions about February 15.

Lot B was treated on March 19. The machine in which

the treatment was conducted was a small sized drier of the type used in some places to remove the excess moisture from grain to prevent spoiling. It consisted of a hopper crossed with vertical perforated partitions in such manner that the contents of the hopper during treatment were in vertical layers about two inches thick and about two inches apart. The vacant spaces were connected with a fan driven by an electric motor which sent an air blast first over a series of electric heating units and then through these spaces. By a system of valves this air could then either be allowed to escape or could be recirculated through the system. The air did not pass intimately among the seed, however.

The seed were dried in this machine for a period of about three hours at temperatures ranging from about 45 degrees to 62 degrees. During this time the weight of the sample was reduced from 5491 gms. to 5345 gms., or 146 gms., which is 2.7 percent of the original weight. Reference to the moisture content (6.47 percent) on April 16, already given, shows that in all probability the treatment removed a little over one-third of the moisture present. These seed were distributed to the respective storage conditions on March 21.

Lot C was treated on April 16, 17 and 18. The seed were dried 42 hours and 20 minutes, in all, at temperatures ranging from 50 degrees to 61 degrees C. except for brief periods for weighing. The seed were weighed at intervals of a few hours and heating coutinued for several hours after the rate of loss from the sample had become less than 0.5 gm. per hour. During this time the weight was reduced from 3129 gms. to 2938 gms. The loss of 191 gms. represents a reduction of 6.1 percent from the original weight. Moisture determinations made before and after treatment showed 6.47 percent and 1.33 percent, respectively, on the basis of gross weight of the sample, thus indicating a loss of only 5.21 per cent. The discrepancy seems rather large but is probably to be ascribed to accidental losses, such as dust, during treatment and to error in sampling. Either figure, however, indicates a removal of most of the uncombined water—practically 80 percent according to the smaller value.

The seed were divided into lots and put in storage on April 21.

Lot D was heated for two hours in a hot oven at approximately 65 degrees C., the maximum temperature reached being 75 degrees for a minute or two and the minimum after the temperature reached the desired point being 59 degrees. The weight was reduced from 4893 gms. to 4790 gms., thus giving a loss of 103 gms. or 2.1 percent of the original weight.

The heating was done in two batches, one on February 17, and one on February 18. The two batches were carefully mixed together before dividing the seed into lots for storage. They were put in storage on February 18.

The seed of lots E and F were delinted, washed with several changes of water, sterilized with a concentrated aqueous solution of mercuric chloride, again washed, and dried. This was done in several portions between February 11 and February 15. These portions were united, mixed well, and then divided into the two lots. Lot E was put in storage on February 18.

Lot F was dried for two hours in the commercial drier on March 18 at a temperature varying from 45 degrees to 58 degrees. The weight was reduced from 3130 gms. to 3054, thus showing a loss of 76 gms., or 2.4 percent.

Table 4 gives the results of the first few determinations made on each sample after the treatments were completed. Table 5 gives the date of the latest test in which anthracnose infection has been found in each of these lots and the date of the next succeeding test for all methods of storage except over calcium chloride. These two dates, in each case, constitute a pair between which the last of the anthracnose infection disappeared, or at least became so small in amount as not to be detected in subsequent determinations.

A study of table 4 reveals the fact that no seed treatment there mentioned eliminated anthracnose from the seed, and none even reduced it appreciably except those in which delinting and surface sterilization figured. This might be true, however, and a treatment still be of value because it would

Table 4.— Effect of Various Treatments on Anthracnose Infection in Toole Cotton Seed

Treatment	Check No Treatment						Dried 3 Hours			Dried 42 hours			Heated 2 Hours				Delinted and Sterilized				Delinted, Sterilized and Dried		
Date (1921)	1-24	2-19	3-19	4-21	4-19, 22	5-27	3-21	4-19, 22	5-27	4-21	4-21	5-30	2-18	3-22	4-19, 22	5-28	2-18,19	3-19	4-19,22	5-28	3-19	4-19,22	5-28
% germination	77.4	§85.1	90.6	90.4	82.7	93.3	†90.0	82.7	88.0	¶89.3	zz2.0	92.0	†90.0	93.3	93.3	89.3	‡93.5	95.4	93.3	86.7	†90.0	96.0	90.7
% seedlings with anthracnose	76.4	81.2	81.2	82.2	80.0	92.0	83.0	81.3	85.3	76.0	46.7	86.7	87.0	87.8	86.7	85.3	16.0	21.3	9.3	4.0	14.0	12.0	9.3
% seedlings without anthracnose	1.0	4.0	10.3	8.2	2.7	1.3	7.0	1.3	2.7	13.3	25.3	5.3	2.0	5.4	6.7	4.0	77.5	73.2	84.0	82.7	76.0	84.0	81.3

* 198 seed.
§ 101 seed.
¶ Sample taken 4-17, during treatment.
z Sample taken 4-19, just after treatment. Infection result probably too low.
† 100 seed.
‡ 200 seed.

Table 5.—Date of Last Test Showing Anthracnose in Toole Cotton Seed and Date of First Test Following for Several Methods of Treatment and Storage

Method of Storage	Check, no treatment		Dried 3 Hours		Dried 42 hours		Delinted, sterilized		Delinted, Sterilized and Dried		Heated 2 Hours	
	Latest date	Next date	Latest date	Next date	Latest date	Next date	Latest date	Next date	Latest date	Next date	Latest date	Next date
Laboratory	5-31-22	6-29-22	5-31-22	6-29-22	5-27-22	6-29-22	5-28-21	7- 8-21	7- 8-21	11-26-21	5-27-22	6-29-22
30° Incubator	2- 6-22	5-12-22	5-12-22	7- 7-22			7-11-21	3- 6-22	7-11-21	3- 6-22	5-12-22	6- 8-22
Over Radiator	4- 3-22	5-25-22	7-12-21	4- 3-22			7-12-21	4- 3-22	7-12-21	4- 3-22	4- 3-22	5-25-22
Out of Doors	1- 5-23	6-23-23	7-15-22	8-16-22	4- 7-22	5-31-22	4- 7-22	5-31-22	4- 7-22	6- 1-22	8-16-22	1- 5-23
Moist Chamber	8- 1-21	11-30-21	8- 1-21	12- 1-21	7-11-21	12- 1-21	8- 1-21	12- 1-21	8- 2-21	12- 1-21	8- 1-21	12- 1-21

hasten the death of the fungus during storage. The data in table 5 throws light on this question. Here again no treatment shows up well in which delinting and sterilizing did not figure. The delinted and sterilized seed were free of disease

Table 6.—Immediate results of various methods of treating and storing anthracnose infected cotton seed.

		Untreated		Three hours in drier		*To approx. constant wt. in drier		Two hours in hot air oven		Average	
		%	No. tests	%	No. tests	%	No. tests	%	No. tests	%	No. tests
Stored in bags in laboratory	% germination	87.8	8	87.5	4	85.7	4	92.4	5	89.1	17
	% seedlings with anthracnose	83.1	8	82.1	4	73.6	4	87.0	5	84.0	17
	Infection %	94.6		93.8		85.9		94.2		94.3	
Stored in 30° incubator	% germination	85.7	4	93.4	3			90.4	4	89.5	11
	% seedlings with anthracnose	80.0	4	86.7	3			83.8	4	83.2	11
	Infection %	93.3		92.8				92.7		93.0	
Stored over radiator	% germination	87.7	4	87.7	3			88.8	4	88.1	11
	% seedlings with anthracnose	82.0	4	77.5	3			80.8	4	80.3	11
	Infection %	93.5		88.4				91.0		91.1	
Stored out of doors	% germination	91.7	4	86.7	3	84.0	2	89.0	4	89.3	11
	% seedlings with anthracnose	82.1	4	72.1	3	76.0	2	81.7	4	79.2	11
	Infection %	89.5		83.2		90.5		91.8		88.7	
Stored in dessicator in laboratory	% germination	86.7	4	85.8	3	72.7	2	80.3	4	84.1	11
	% seedlings with anthracnose	75.3	4	66.2	3	56.0	2	61.5	4	67.8	11
	Infection %	86.9		77.2		77.0		76.6		80.6	
Stored in dessicator in 30° incubator	% germination	86.6	4	84.2	3			74.3	4	81.4	11
	% seedlings with anthracnose	71.8	4	63.2	3			57.6	4	64.3	11
	Infection %	82.9		75.0				77.5		79.0	
Average	% germination	87.7	28	87.5	19	82.0	8	86.1	25	87.1	72
	% seedlings with anthracnose	79.6	28	75.0	19	69.7	8	75.8	25	77.1	72
	Infection %	90.8		85.7		85.0		88.0		88.5	

* This column not considered in the average in the right hand column.

Note.—The "infection %" is the same as "% seedlings with anthracnose" except that it is calculated on the number of seeds which germinated instead of the number planted.

in most cases by the second spring after ginning and they were the only seed that were free.

Table 6 gives in more detailed form the immediate results of the different treatments. Here are given the percentages of germination and infection and the percentage of infection based on that of germination for the first summer of storage, together with the number of determinations on which each figure is based. It will be noted that in general there is a small decrease in the amount of infection in the treated lots. It is scarcely to be considered significant, though; and certainly it is of no consequence from the standpoint of control.

It seems clear that no ordinary method of drying or dry heating cotton seed previous to storage has any practical value for ridding the seed of anthracnose. On the other hand delinting and sterilizing (and probably delinting without further sterilization) does have real merit. It eliminates infection on the outside of the seed and permits the seed to become clean of anthracnose sooner in storage.

These figures, so far as the immediate results of delinting and sterilizing are concerned, are verified by similar tests on the Cook cotton seed in 1920. Table 7 gives these results. When it is considered that the infection was originally somewhere around 60 percent it will be seen that a large deduction was accomplished by the treatment. It is worthy of remark here, also, that the plantlets produced in the tubes from

Table 7.—Percentage of Anthracnose in Delinted and Sterilized Cook Cotton Seed, 1920

Date	No. Seeds	% Germination	% Seedlings with Anthracnose	% Seedlings without Anthracnose
Apr. 5	47	83.0	4.3	78.7
Apr. 12	50	86.0	8.3	77.1
June 4	75	77.3	6.7	70.7
July 16	75	69.3	5.3	64.0*
Aug. 19	75	80.0	6.7	72.0
Sept. 17	75	82.7	1.3	81.3

* Dried in vacuum 60 hrs.

such seeds are remarkably clean and free from all fungi and that the seed germinate more promptly and come up more uniformly than ordinary seed under adverse conditions in the field. It must not be forgotten, however, that this treatment, though of value, is in no sense a specific. It is not a thing that will eradicate the disease at a single stroke.

The Effect of Various Storage Conditions

The percentage of anthracnose infection at various dates under the different combinations of preliminary treatment and conditions of storage are shown in detail in tables 8, 9, 10, 11, 12, and 13, and figures 4, 5, 6, 7, 8, and 9. Table 5, already mentioned, gives a summary of these results on the basis of the time taken for the seed to become free from the disease.

A consideration of these tables and curves shows that after the seed were nearly a year old a steady and rapid decrease in the amount of anthracnose infection began for all methods of storage tried except in the extremely dry atmosphere existing over calcuim chloride. This decrease was initiated sooner and proceeded more rapidly in the lots stored in a moist atmosphere than in any other. No determination on any seed stored under these conditions showed infection after August 2 of the first year. About the time the anthracnose infection disappeared, however, the lots became musty; and the seed soon failed to germinate. This method of storage, as was suspected beforehand, is not suited to practical purposes.

The method of storage next most fatal to the fungus seems to have been that over the radiator, although its advantage does not seem to be very great over the other ordinary methods. The least effective of the ordinary methods of storage was out of doors but under a roof. With only one exception, that of the sample dried for 42 hours, the fungus persisted longer under these conditions than in any of the others. This should explain in a measure the circumstance referred to earlier in this bulletin that long storage of infected seed by farmers is not always as satisfactory in freeing it

Table 8.—Germination percentage, infection percentage, and percentage not infected of untreated Toole cotton seed at various dates after various methods of storage.

1. Stored in bags in laboratory

Date	1921									1922					1923	1924
	1–24	2–19	3–19	4–21	4–19 & 4–22	5–27	6–25	7–8	11–25	3–21	5–31	6–29	8–3	12–20	2–17	2–12
% germination	*77.4	†85.1	90.6	90.4	82.7	93.3	89.3	93.3	93.3	93.3	94.7	94.7	85.3	94.5	93.3	97.3
% seedlings with anthracnose	76.4	81.2	81.2	82.2	80.0	92.0	84.0	88.0	12.0	5.3	1.3	0.0	0.0	0.0	0.0	0.0
% seedlings without anthracnose	1.0	4.0	10.3	8.2	2.7	1.3	5.3	5.3	81.3	88.0	93.3	94.7	85.3	94.5	93.3	97.3

2. Stored in 30° incubator

Date	1921				1922						1923
	3–23	4–20 & 4–23	5–28	7–8	2–6	5–12	7–7	7–8	8–9	12–20	2–19
% germination	77.3	86.7	90.7	88.0	81.3	90.7	88.0	89.3	82.7	84.0	94.7
% seedlings with anthracnose	72.0	77.3	89.3	81.3	17.3	0.0	0.0	0.0	0.0	0.0	0.0
% seedlings without anthracnose	5.3	9.3	1.3	6.7	64.0	90.7	88.0	89.3	82.7	84.0	94.7

3. Stored over radiator

Date	1921				1922				1923	
	3–23	4–20 4–23	5–30	7–12	4–3	5–25	6–24	8–2	1–4	2–20
% germination	84.0	86.7	89.3	90.7	78.7	90.7	84.6	86.7	86.7	81.3
% seedlings with anthracnose	77.3	78.7	89.3	82.7	1.3	0.0	0.0	0.0	0.0	0.0
% seedlings without anthracnose	6.7	8.0	0.0	8.0	77.3	90.7	84.6	86.7	86.7	81.3

4. Stored out of doors

Date	1921					1922				1923		1924
	4–7	5–13	6–17	7–29	12–6	4–7	5–31	7–13	8–16	1–5	6–23	2–12
% germination	96.0	89.3	92.1	89.3	90.7	94.7	90.7	93.3	88.0	89.3	86.7	93.3
% seedlings with anthracnose	92.0	73.3	84.2	78.7	36.0	28.8	13.3	12.0	0.0	2.7	0.0	0.0
% seedlings without anthracnose	4.0	16.0	7.9	10.7	54.7	65.8	77.3	81.3	88.0	86.7	86.7	93.3

5. Stored in moist chamber

Date	1921					1922		
	4–7	5–13	6–17	8–1	11–30	4–13	5–13	
% germination	92.0	87.8	92.0	68.0	1.3	‡0.0	0.0	Discarded
% seedlings with anthracnose	72.0	77.0	58.7	6.7	0.0	0.0	0.0	7-15-22
% seedlings without anthracnose	20.0	10.8	33.3	61.3	1.3	0.0	0.0	

6. Stored in dessicator in laboratory

Date	1921					1922				1923		1924
	4–7	5–14	6–20	8–2	2–24	4–25	6–8	7–27	8–31	2–7	6–16	2–13
% germination	91.0	85.3	88.0	82.4	57.3	74.7	77.3	90.7	81.3	85.3	77.3	90.7
% seedlings with anthracnose	89.7	72.0	72.0	67.6	48.0	62.7	45.3	80.0	25.3	66.7	69.3	69.3
% seedlings without anthracnose	1.3	13.3	16.0	14.9	9.3	12.0	32.0	10.7	56.0	18.7	8.0	21.3

7. Stored in dessicator in 30° incubator

Date	1921				1922			1923	1924
	4–9	5–14	6–30	8–3	3–6	7–19	8–30	2–7	6–9	3–5
% germination	93.3	78.7	91.8	82.4	57.3	85.3	86.1	92.0	81.3	86.7
% seedlings with anthracnose	78.7	64.0	76.7	67.6	50.7	72.0	67.1	78.7	60.0	58.7
% seedlings without anthracnose	14.7	14.7	15.1	14.9	6.7	13.3	19.0	13.3	21.3	28.0

* 198 seeds used.
† 101 seeds used.
‡ 25 seeds used.

Table 9.—Germination percentage, infection percentage, and percentage not infected of Toole cotton seed dried three hours in a commercial drier, stored in various ways, and tested at various dates.

1. Stored in bags in laboratory

Date	1921					1922					1923
	3–21	4-19 & 4–22	5–27	7–8	11–26	3–21	5–31	6–29	8–3	12–20	2–17
% germination	*90.0	82.7	88.0	89.3	94.7	90.7	96.0	93.3	86.7	81.3	89.3
% seedlings with anthracnose	83.0	81.3	85.3	78.7	6.7	13.3	1.3	0.0	0.0	0.0	0.0
% seedlings without anthracnose	7.0	1.3	2.7	10.7	88.0	77.3	94.7	93.3	86.7	81.3	89.3

2. Stored in 30° incubator

Date	1921			1922						1923
	4-20 & 4–23	5–28	7–11	3–6	5–12	7–7	7–8	8–9	12–21	2–19
% germination	92.1	96.0	92.0	84.0	92.0	84.0	94.7	92.0	86.7	89.3
% seedlings with anthracnose	82.9	93.3	84.0	14.7	1.3	0.0	0.0	0.0	0.0	0.0
% seedlings without anthracnose	9.2	2.7	8.0	69.3	90.7	84.0	94.7	92.0	86.7	89.3

3. Stored over radiator

Date	1921			1922				1923	
	4-20 & 4–24	5–30	7–12	4–3	5–25	6–27	8–2	1–4	2–20
% germination	78.7	85.7	89.3	93.3	90.7	90.7	85.3	84.0	88.0
% seedlings with anthracnose	88.0	81.8	72.0	0.0	0.0	0.0	0.0	0.0	0.0
% seedlings without anthracnose	9.3	3.9	17.3	93.3	90.7	90.7	85.3	84.0	88.0

4. Stored out of doors

Date	1921				1922				1923	
	5–13	6–17	7–29	12–7	4–7	5–31	7–15	8–16	1–5	6–23
% germination	81.3	92.1	86.7	82.7	93.3	92.0	85.3	88.0	90.7	88.0
% seedlings with anthracnose	73.3	80.3	62.7	9.3	9.3	1.3	1.3	0.0	0.0	0.0
% seedlings without anthracnose	8.0	11.8	24.0	73.3	84.0	90.7	84.0	88.0	90.7	88.0

5. Stored in moist chamber

Date	1921				1922	
	5–13	6–17	8–1	12–1	5–13	
% germination	85.3	89.3	86.7	1.3	1.3	Discarded
% seedlings with anthracnose	77.3	60.0	12.0	0.0	0.0	June 15, 1922
% seedlings without anthracnose	8.0	29.3	74.7	1.3	1.3	

6. Stored in dessicator in laboratory

Date	1921			1922					1923		1924
	5–14	6–20	8–2	2–21	4–24	6–8	7–27	8–31	2–9	6–16	2–13
% germination	84.0	81.3	92.0	45.3	53.3	77.3	74.7	81.1	†80.0	73.3	86.7
% seedlings with anthracnose	66.7	61.3	70.7	34.7	44.0	56.6	58.7	60.8	58.6	57.3	60.0
% seedlings without anthracnose	17.3	20.0	21.3	10.7	9.3	20.8	16.0	20.3	21.4	16.0	26.7

7. Stored in dessicator in 30° incubator

Date	1921			1922				1923		1924
	5–14	6–20	8–3	3–6	5–31	7–19	8–30	2–7	6–9	3–5
% germination	84.2	85.6	82.7	†57.1	94.7	78.7	90.5	90.7	85.3	88.0
% seedlings with anthracnose	57.9	73.0	58.7	45.7	73.3	61.3	75.7	77.3	68.0	58.7
% seedlings without anthracnose	26.3	12.2	24.0	11.4	21.3	17.3	14.9	13.3	17.3	29.3

* 100 seeds used.
† 70 seeds used.

Table 10.—Germination percentage, infection percentage, and percentage not infected of Toole cotton seed dried approximately to constant weight in a commercial drier, stored in various ways, and tested at various dates.

1. Stored in bag in laboratory

Date	1921					1922					1923
	4–21	4–21	5–30	7–8	11–25	3–21	5–27	6–29	8–3	12–20	2–17
% germination	*89.3	†72.0	92.0	89.3	92.0	90.7	95.9	84.0	89.5	90.7	88.0
% seedlings with anthracnose	76.0	46.7	86.7	84.0	0.0	13.3	1.4	0.0	0.0	0.0	0.0
% seedlings without anthracnose	13.3	25.3	5.3	5.3	92.0	77.3	94.6	84.0	89.5	90.7	88.0

4. Stored out of doors

Date	1921		1922					1923	
	5–30	7–11	12–7	4–7	5–31	7–15	8–16	1–5	6–23
% germination		82.7	85.3	85.3	89.3	88.0	77.3	77.3	82.7	88.0
% seedlings with anthracnose		78.7	73.3	1.3	2.7	0.0	0.0	0.0	0.0	0.0
% seedlings without anthracnose		4.0	12.0	84.0	86.7	88.0	77.3	77.3	82.7	88.0

5. Stored in moist chamber

Date	1921			1922		
	5–30	7–11	12–1	4–13	5–13	
% germination	88.0	82.7	38.7	‡0.0	12.0	Discarded
% seedlings with anthracnose	73.3	54.7	0.0	0.0	0.0	June 15, 1922
% seedlings without anthracnose	14.7	28.0	38.7	0.0	12.0	

6. Stored in dessicator in laboratory

Date	1921	1922						1923		1924
	5–30	7–11	2–21	4–26	6–8	7–27	8–31	2–7	6–16	2–13
% germination	§68.0	77.3	61.3	61.3	81.6	80.0	85.3	72.0	78.7	70.7
% seedlings with anthracnose	57.3	54.7	54.7	54.7	65.8	60.0	57.3	57.3	50.7	50.7
% seedlings without anthracnose		22.7	6.7	6.7	15.8	20.0	28.0	14.7	28.0	20.0

* Sample taken 4-17, during treatment.

† Sample taken 4-19, immediately after close of treatment.

‡ 25 seeds used.

§ Infection figure perhaps too low, as not all the seedlings are accounted for in the original record of this test.

from anthracnose as is storage in the laboratory. The reason for the condition is not clear, however, since the laboratory is drier than the open balcony. In the other experiments discussed here extreme dampness tended to the rapid destruction of the parasite and extreme dryness to long life.

A somewhat anomalous result, but one which is uniform and consistent enough not easily to be ascribed to chance, is that the undelinted samples stored in very dry air underwent an initial slight reduction in the amount of the fungus present. This point can be observed by an examination of the figures and is brought out especially by table 6. It will also be noted from an examination of the table that the germination percent was also decreased. That this decrease in germination does not account for all of the decrease in the number of infected seedlings, however, is apparent from the fact that there is a decrease in the percentage of infected seedlings when figured on the basis of the number of seeds which germinated. A similar test with the Webber 82 seed (table 14 and fig. 10), to be discussed later, did not duplicate this result. Just what significance is to be attached to it is not clear, particularly in view of the failure of the two tests to agree.

The most conspicuous result of this experiment, therefore, is the fact that the storage of infected seed in a uniformly very dry atmosphere extends the life of the fungus very greatly. The amount of infection still present March 1, 1924, more than three years after harvest was still very high (b).

The result is verified by the experiment carried out in 1923. A number of observations during the course of the work just discussed, particularly that storage over a radiator seemed to have some merit for destroying the fungus and that the fungus died most rapidly in a moist atmosphere, suggested that storing the seed under alternating extremes of condition

(b) An additional test on the lots of seed stored in dessicators was started on March 7, 1925. This test was unfortunately terminated and the stored seed destroyed by the fire which destroyed the Agricultural Hall in the early morning of April 2. The same fire consumed all records of the test, so that no statistical report on it can be made. The results showed, however, that the amount of infection in these samples was still high, thus showing its persistence for more than four years after harvest.

Table 11.—Germination percentage, infection percentage, and percentage not infected of Toole cotton seed heated two hours at approximately 65° C., stored in various ways, and tested at various dates.

1. Stored in bag in laboratory

Date	1921						1922					1923
			4-19 &									
	2–18	3–22	4–22	5–28	7–8	11–26	3–22	5–27	6–29	8–3	12–21	2-17
% germination	*90.0	93.3	93.3	89.3	96.0	96.0	94.7	96.0	93.3	88.0	88.0	91.9
% seedlings with anthracnose	87.0	87.8	86.7	85.3	88.0	1.3	5.3	1.3	0.0	0.0	0.0	0.0
% seedlings without anthracnose	3.0	5.4	6.7	4.0	8.0	94.7	89.3	94.7	93.3	88.0	88.0	91.9

2. Stored in 30° incubator

Date	1921				1922						1923
		4-20 &									
	3–23	4–23	5–28	7–11	3–6	5–12	6–8	7–8	8–9	12–21	2–20
% germination	82.7	90.7	96.0	†92.3	90.7	93.3	90.7	94.7	88.0	86.7	90.7
% seedlings with anthracnose	74.7	86.7	89.3	84.6	26.7	1.3	0.0	0.0	0.0	0.0	0.0
% seedlings without anthracnose	8.0	4.0	6.7	7.7	64.0	92.0	90.7	94.7	88.0	86.7	90.7

3. Stored over radiator

Date	1921				1922				1923	
	3–23	4–25	5–30	7–12	4–3	5–25	6–24	8–2	1–4	2–20
% germination	88.5	86.8	89.3	90.7	92.0	96.0	92.2	92.0	93.3	86.7
% seedlings with anthracnose	78.7	81.6	86.7	76.0	2.7	0.0	0.0	0.0	0.0	0.0
% seedlings without anthracnose	9.8	5.3	2.7	14.7	89.3	96.0	92.2	92.0	93.3	86.7

4. Stored out of doors

Date	1921					1922				1923		1924
	4–7	5–13	6–17	7–29	12–8	4–7	6–1	7–17	8–16	1–5	6–23	2–12
% germination	92.0	85.3	89.3	89.3	92.0	93.3	92.0	90.7	93.3	89.3	89.3	86.7
% seedlings with anthracnose	86.7	82.7	86.7	70.7	40.0	29.3	8.0	1.3	1.3	0.0	0.0	0.0
% seedlings without anthracnose	5.3	2.7	2.7	18.7	52.0	64.0	84.0	89.3	92.0	89.3	89.3	86.7

5. Stored in moist chamber

Date	1921				1922	
	4–7	5–14	6–18	8–1	12–1	5–13
% germination	94.7	74.7	88.2	77.3	2.7	0.0
% seedlings with anthracnose	88.0	72.0	55.3	8.0	0.0	0.0
% seedlings without anthracnose	6.7	2.7	32.9	69.3	2.7	0.0

Discarded June 15, 1922

6. Stored in dessicator in laboratory

Date	1921				1922					1923		1924
	4–8	5–14	6–20	8–2	2–21	4–25	6–14	7–27	8–31	2–7	6–16	2–14
% germination	77.3	84.0	73.0	86.7	61.3	52.0	81.3	76.0	81.3	72.0	84.0	88.0
% seedlings with anthracnose	61.3	60.0	56.8	68.0	54.7	49.3	49.3	53.3	60.0	61.3	62.7	66.7
% seedlings without anthracnose	15.0	24.0	16.2	18.7	6.7	2.7	32.0	22.7	21.3	10.7	21.3	21.3

7. Stored in dessicator in 30° incubator

Date	1921				1922				1923		1924
	4–10	5–16	6–20	8–3	3–7	6–1	7–18	8–30	2–7	6–11	3–6
% germination	70.3	61.3	78.7	86.7	44.0	75.3	72.4	71.4	78.7	68.0	80.0
% seedlings with anthracnose	59.5	42.7	60.0	68.0	38.7	60.3	52.6	57.1	62.7	56.0	46.7
% seedlings without anthracnose	10.8	18.7	18.7	18.7	5.3	15.1	19.7	14.3	16.0	12.0	33.3

* 100 seeds used.
† 65 seeds used.

Table 12.—Germination percentage, infection percentage, and percentage not infected of delinted and sterilized Toole cotton seed at various dates after various methods of storage.

1. Stored in bag in laboratory

Date	1921						1922					1923
	2-18 & 2-19	3-19	4-19 & 4-22	5-28	7-8	11-26	3-22	5-27	6-29	8-3	12-21	2-17
% germination	*93.5	95.4	93.3	86.7	94.7	97.3	97.3	94.7	93.3	92.0	92.0	97.3
% seedlings with anthracnose	16.0	21.3	9.3	4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
% seedlings without anthracnose	77.5	73.2	84.0	82.7	94.7	97.3	97.3	94.7	93.3	92.0	92.0	97.3

2. Stored in 30° incubator

Date	1921			1922							1923
	3-23	4-20 & 4-23	5-28	7-11	3-6	5-12	6-8	7-8	8-9	12-21	2-20
% germination	81.3	96.0	89.3	88.0	89.3	94.6	93.3	93.3	87.8	94.7	96.0
% seedlings with anthracnose	10.7	10.7	12.0	8.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
% seedlings without anthracnose	70.7	85.3	77.3	80.0	89.3	94.6	93.3	93.3	87.8	94.7	96.0

3. Stored over radiator

Date	1921				1922				1923	
	3-23	4-20 & 4-24	5-30	7-12	4-3	5-25	6-24	8-2	1-4	2-20
% germination	85.3	93.3	89.3	90.7	94.7	92.0	94.7	88.0	93.3	89.3
% seedlings with anthracnose	12.0	13.3	4.0	4.0	0.0	0.0	0.0	0.0	0.0	0.0
% seedlings without anthracnose	73.3	80.0	85.3	86.7	94.7	92.0	94.7	88.0	93.3	89.3

4. Stored out of doors

Date	1921					1922				1923	
	4–7	5–13	6–17	7–29	12–7	4–7	5–31	7–15	8–16	1–5	6–23
% germination	93.3	89.3	89.3	93.3	90.7	92.0	86.7	93.3	92.1	89.3	80.0
% seedlings with anthracnose	10.7	20.0	5.3	16.0	0.0	2.7	0.0	0.0	0.0	0.0	0.0
% seedlings without anthracnose	82.7	69.3	84.0	77.3	90.7	89.3	86.7	93.3	92.1	89.3	80.0

5. Stored in moist chamber

Date	1921					1922	
	4–7	5–13	6–18	8–1	12–1	5–13	
% germination	93.3	90.7	88.0	69.3	6.7	0.0	Discarded
% seedlings with anthracnose	14.7	6.7	8.0	5.3	0.0	0.0	June 15,
% seedlings without anthracnose	78.7	84.0	80.0	64.0	6.7	0.0	1922

6. Stored in dessicator in laboratory

Date	1921				1922					1923		1924
	4–7	5–14	6–20	8–2	2–21	5–11	6–8	7–27	8–31	2–7	6–16	2–13
% germination	90.7	85.3	96.0	94.7	94.7	89.3	90.7	88.0	97.3	97.3	89.3	93.3
% seedlings with anthracnose	12.2	16.0	10.7	10.7	5.3	12.0	4.0	6.7	8.0	5.3	4.0	6.7
% seedlings without anthracnose	78.4	69.3	85.3	84.0	89.3	77.3	86.7	81.3	89.3	92.0	85.3	86.7

7. Stored in dessicator in 30° incubator

Date	1921				1922				1923		1924
	4–10	5–14	6–20	8–2	3–6	5–11	7–18	8–30	2–7	6–9	3–5
% germination	90.4	93.2	93.3	96.0	90.7	96.0	84.0	96.0	92.0	93.3	94.7
% seedlings with anthracnose	13.7	8.1	13.3	6.7	9.3	6.7	8.0	2.7	5.3	12.0	4.0
% seedlings without anthracnose	76.7	85.1	80.0	89.3	81.3	89.3	76.0	93.3	86.7	81.3	90.7

* 200 seeds used.

Table 13.—Germination percentage, infection percentage, and percentage not infected of delinted and sterilized Toole cotton seed dried two hours in a commercial drier, stored in various ways, and tested at various dates.

1. Stored in bag in laboratory

Date	1921					1922					1923
	3–19	4-19 & 4–22	5–28	7–8	11–26	3–22	5–27	6–29	8–3	12–21	2–17
% germination	*90.0	96.0	90.7	94.7	96.0	90.7	97.3	92.0	92.0	92.0	91.9
% seedlings with anthracnose	14.0	12.0	9.3	4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
% seedlings without anthracnose	76.0	84.0	81.3	90.7	96.0	90.7	97.3	92.0	92.0	92.0	91.9

2. Stored in 30° incubator

Date	1921			1922						1923
	4-20 & 4–22	5–28	7–11	3–6	5–12	6–8	7–8	8–9	12–21	2–20
% germination	97.3	94.7	92.0	97.3	92.0	96.0	94.7	92.0	89.3	90.7
% seedlings with anthracnose	12.0	6.7	2.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
% seedlings without anthracnose	85.3	88.0	89.3	97.3	92.0	96.0	94.7	92.0	89.3	90.7

3. Stored over radiator

Date	1921			1922				1923	
	4–24	5–30	7–12	4–3	5–25	6–26	8–2	1–4	2–20
% germination	86.7	93.3	89.3	88.0	97.3	93.3	93.3	97.3	89.3
% seedlings with anthracnose	9.3	4.0	4.0	0.0	0.0	0.0	0.0	0.0	0.0
% seedlings without anthracnose	77.3	89.3	85.3	88.0	97.3	93.3	93.3	97.3	89.3

4. Stored out of doors

Date	1921				1922				1923	
	5–13	6–17	7–30	12–8	4–7	6–1	7–15	8–16	1–15	6–23
% germination	96.0	86.5	90.7	92.0	93.3	94.7	86.7	89.3	85.3	88.0
% seedlings with anthracnose	8.0	6.8	5.3	6.7	2.7	0.0	0.0	0.0	0.0	0.0
% seedlings without anthracnose	88.0	79.7	85.3	85.3	90.7	94.7	86.7	89.3	85.3	88.0

5. Stored in moist chamber

Date	1921				1922	
	5–14	6–18	8–2	12–1	5–13	
% germination	85.3	92.0	80.0	0.0	0.0	Discarded
% seedlings with anthracnose	4.0	8.0	2.7	0.0	0.0	June 15,
% seedlings without anthracnose	81.3	84.0	77.3	0.0	0.0	1922

6. Stored in dessicator in laboratory

Date	1921			1922					1923		1924
	5–14	6–20	8–2	2–21	4–25	6–14	7–27	8–31	2–7	6–16	2–13
% germination	92.0	89.3	93.3	85.3	82.7	88.2	77.3	92.0	93.3	97.3	96.0
% seedlings with anthracnose	8.0	12.0	10.7	12.0	2.7	6.6	5.3	9.3	6.7	2.7	8.0
% seedlings without anthracnose	84.0	77.3	82.7	73.3	80.0	81.6	72.0	82.7	86.7	94.7	88.0

.7 Stored in dessicator in 30° incubator

Date	1921			1922				1923		1924
	5–16	6–20	8–3	3–7	5–11	7–18	8–30	2–7	6–9	3–6
% germination	79.7	93.3	92.0	92.0	78.7	93.3	92.0	92.0	97.3	97.3
% seedlings with anthracnose	8.1	12.0	5.3	12.0	4.0	5.3	5.3	6.7	2.7	4.0
% seedlings without anthracnose	71.6	81.3	86.7	80.0	74.7	88.0	86.7	85.3	94.7	93.3

* 100 seeds used .

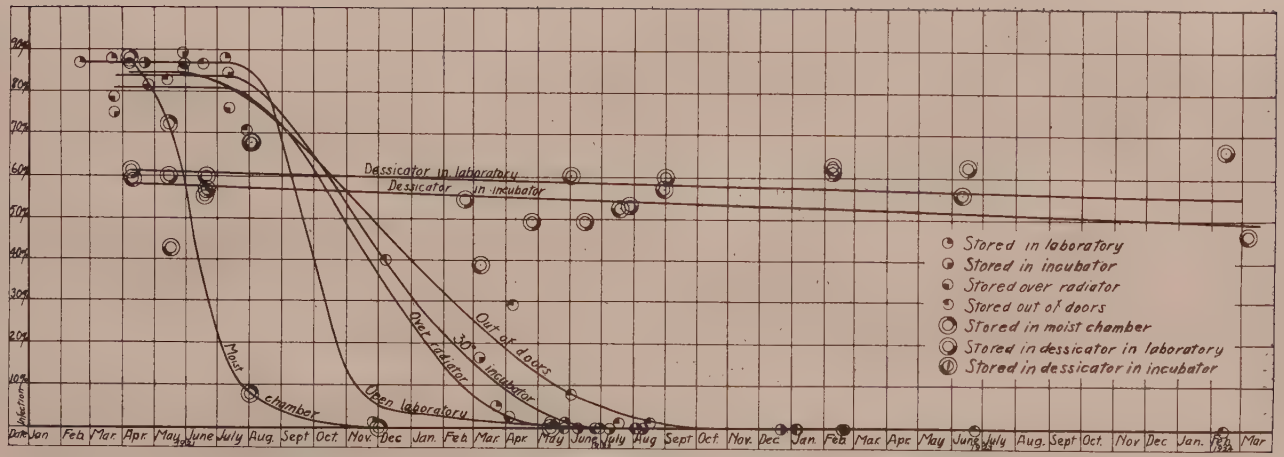

Fig. 4.—Curves showing the rate of decrease of the anthracnose fungus in untreated Toole cotton seed under various methods of storage.

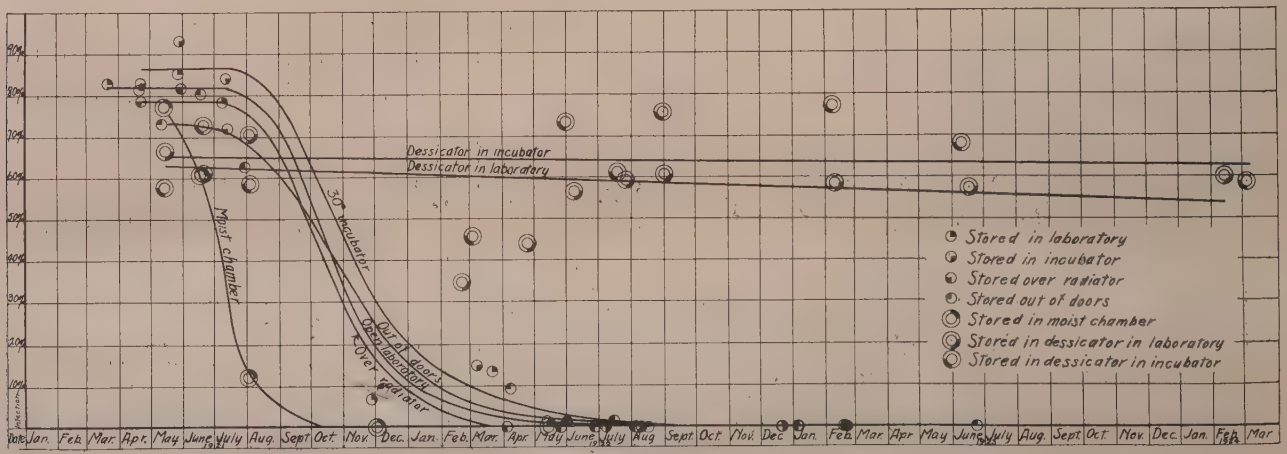

Fig. 5.—Curves showing the rate of decrease of the anthracnose fungus in Toole cotton seed dried three hours in a commercial drier and stored in various ways.

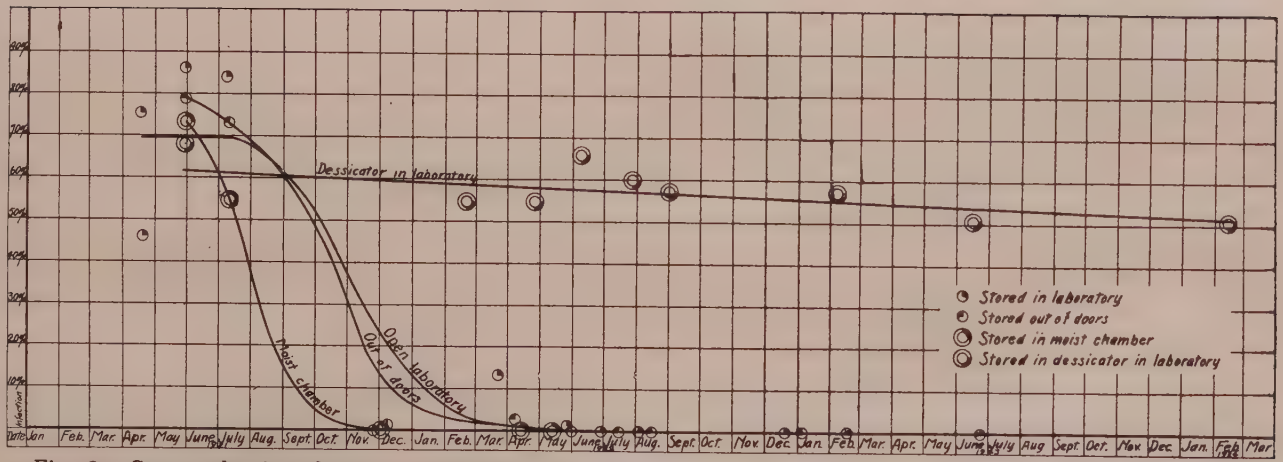

Fig. 6.—Curves showing the rate of decrease of the anthracnose fungus in Toole cotton seed dried approximately to constant weight and stored in various ways.

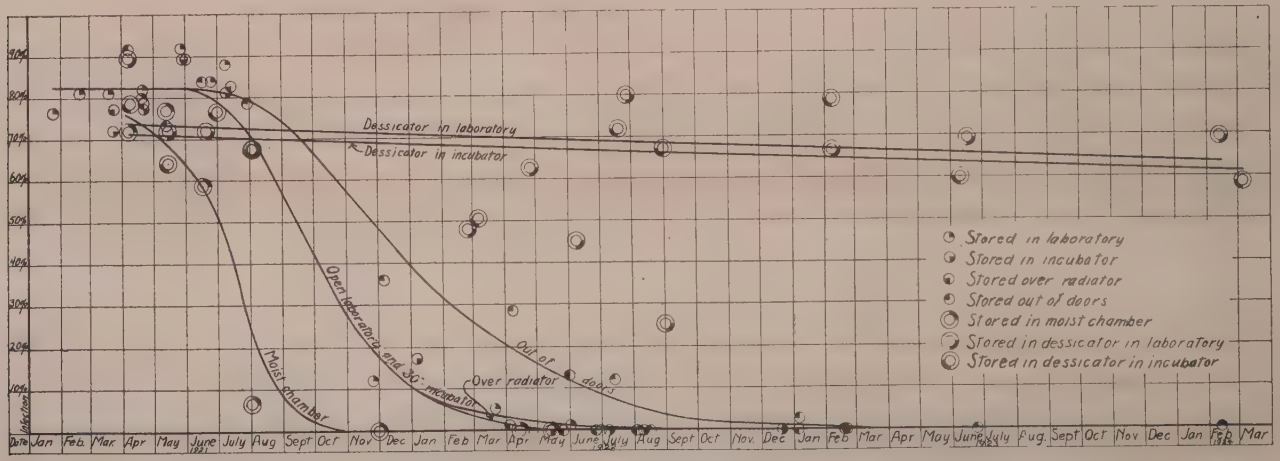

Fig. 7.—Curves showing the rate of decrease of the anthracnose fungus in Toole cotton seed heated to 65 C. for two hours in a hot oven and stored in various ways.

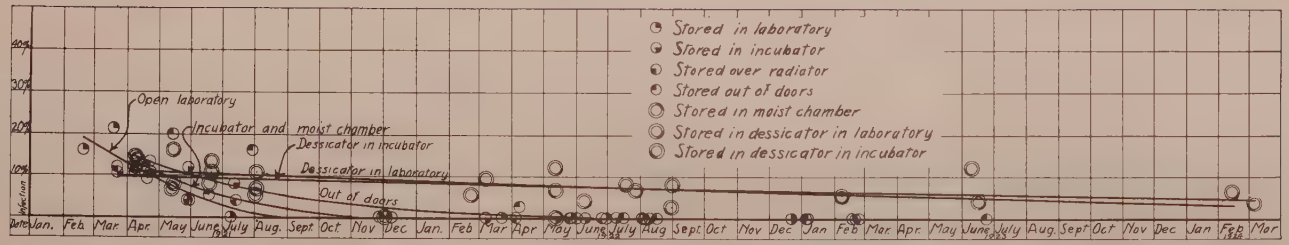

Fig. 8.—Curves showing the rate of decrease of the anthracnose fungus in Toole cotton seed delinted and sterilized and stored in various ways.

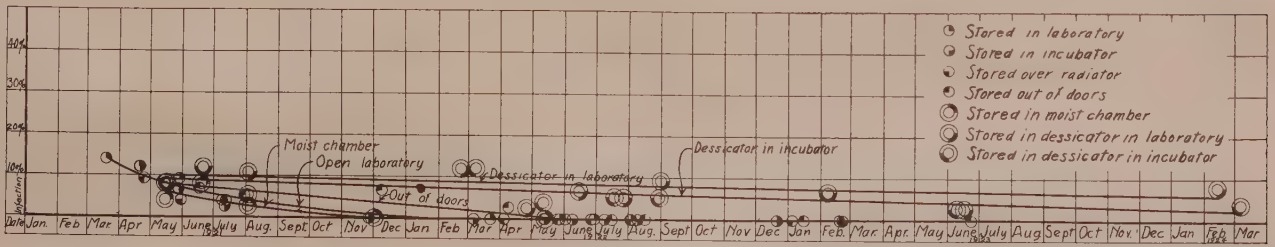

Fig. 9.—Curves showing the rate of decrease of the anthracnose fungus in Toole cotton seed delinted, sterilized, and dried two hours in a commercial drier, and stored in various ways.

Table 14.—Effect of alternating moist and dry air on anthracnose in stored Webber 82 cotton seed

Check, stored in open laboratory

Date	1923																						1924	
	2–7	2–21	3–7	3–24	4–7	4–18	5–2	5–16	5–13	6–13	6–27	7–11	7–25	8–8	8–22	9–5	9–20	10–3	10–18	11–3	11–14	1–24	2–22
% germination	86.5	85.3	97.3		96.0	94.7	90.7	94.6	96.0	93.3	96.0	96.0	97.3	94.7	98.7	98.7	98.7	94.7	*95.7	94.7	94.7	98.7	97.3
% seedlings with anthracnose	62.2	57.3	62.7		53.3	60.0	57.3	51.4	58.7	54.7	38.7	70.7	53.3	30.7	14.7	5.3	9.3	1.3	1.4	2.7	0.0	0.0	0.0
% seedlings without anthracnose	24.3	28.0	34.7		42.7	34.7	33.3	43.2	37.3	38.7	57.3	25.3	44.0	64.0	84.0	93.3	89.3	93.3	94.3	92.0	94.7	98.7	97.3

Alternated between dessicator and moist chamber

																9–19		10–17					
% germination	85.3	86.7	89.3	§78.0	93.3	76.7	92.0	*97.1	98.7	97.3	92.0	97.3	89.3	90.7	90.7	88.0	93.3	82.7	70.7	90.7	80.0	76.0	73.3
% seedings with anthracnose	56.0	44.0	11.0	32.0	56.0	23.3	49.3	42.9	56.0	42.7	26.7	38.7	9.3	4.0	14.7	5.3	5.3	2.7	0.0	0.0	1.3	0.0	0.0
% seedlings without anthracnose	29.3	42.7	15.3	46.0	37.3	53.4	42.7	54.3	42.7	54.7	65.3	58.7	80.0	86.7	76.0	82.7	88.0	80.0	79.7	90.7	78.7	76.0	73.3

Stored in dessicator

																						1–25	
% germination	89.3	98.7	90.7		86.7	81.3	86.7	98.7	94.7	78.7	93.2	92.0	88.0	90.7	93.3	89.3	84.0	90.7	94.7	94.7	86.7	90.7	88.0
% seedlings with anthracnose	65.3	61.3	57.3		32.0	50.7	53.3	50.7	64.0	48.0	47.3	72.0	49.3	64.0	73.3	68.0	61.3	54.7	52.0	65.3	46.7	50.7	50.7
% seedlings without anthracnose	24.0	37.3	33.3		54.7	30.7	33.3	48.0	30.7	30.7	45.9	20.0	38.7	26.7	20.0	21.3	29.7	36.0	42.7	29.3	40.0	40.0	37.3

*70 seeds only
§50 seeds only

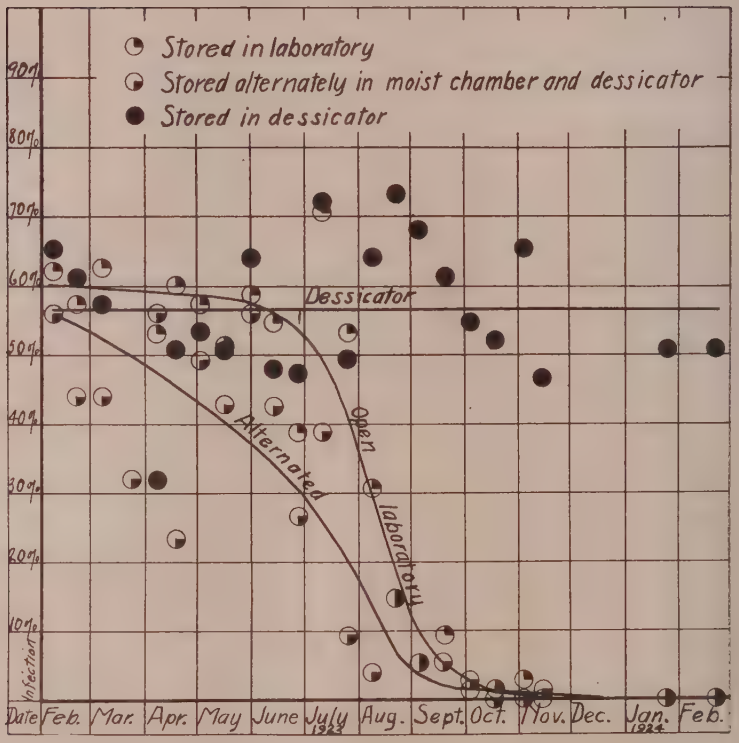

Fig. 10.—Curves showing the rate of decrease of the anthracnose fungus in Webber 82 cotton seed stored in different ways.

might be advantageous. Accordingly, a sample of the Webber 82 seed was divided into three lots and stored as follows: (1) In a paper bag in the open laboratory to serve as a check, (2) in a paper bag alternated two weeks at a time between a chamber containing a dish of water and a dessicator containing calcuim chloride; and (3) in a paper bag stored permanently in a dessicator over calcium chloride.

The results of this study are tabulated in detail in table 14 and presented graphically in figure 10. Here the extreme persistence of the fungus under dry conditions is again brought forcibly to attention (c).

Whatever may be the bearing of this behavior on the question of the cause of the death of the anthracnose fungus and spores in cotton seed in storage one thing seems quite certain, and that is that the death of the organism is due in no sense simply to drying. A thoroughly dry atmosphere is precisely the one in which the infection persists longest.

Under conditions of alternation of very moist and very dry conditions the fungus appears to have died out somewhat more rapidly than under ordinary laboratory storage, especially early in the period of storage. However, complete freedom from infection did not come much sooner, if any. The only thing that makes the result seem at all doubtful for the early part of the storage period is the fact that the first test, made on February 7 when the experiment started, showed less infection in the alternated lot than in the other two. As the seed were well mixed and then carefully divided into the three lots, however, the lowness of this particular test is more reasonably to be ascribed to chance variation in the sampling than to actual lower infection of the lot.

Only once was any mustiness noted, and that was apparently checked effectually by the next turn in the dessicator.

The evidence seems to be, therefore, that this type of storage will reduce anthracnose infection more rapidly than ordinary laboratory storage early in the storage period but not so rapidly as continuous storage in a moist atmosphere. It does not appear that it will make the date of complete freedom come much earlier than will ordinary laboratory storage.

Effect of Sunning Seed on the Life of the Fungus

An experiment was conducted with some of the Toole seed, beginning in June, 1921, to determine whether or not sunning infected seed would have a beneficial effect in ridding it of anthracnose, and if so, whether or not such treatment would be worth while from a practical standpoint. Two lots

---

(c) A further test of the dessicator stored seed of this lot was started on March 7, 1925, as described above for the Toole seed. The results in this case showed heavy infection still persisting more than two years after harvest.

Table 15.—Effect of sunning on anthracnose infection in Toole cotton seed.

Untreated seed

Date	1921				1922				
	5–27	6–25	7–8	11–25	3–21	5–31	6–29	8–3	12–20
% germination	93.3	†89.3	93.3	93.3	93.3	94.7	94.7	85.3	94.5
% seedlings with anthracnose	92.0	84.0	88.0	12.0	5.3	1.3	0.0	0.0	0.0
% seedlings without anthracnose	1.3	5.3	5.3	81.3	88.0	93.3	94.7	85.0	94.5

Sunned seed No. 1

Date	1921						1922					
	6–25	7–2	7–9	7–16	8–8	10–26	2–25	4–15	6–5	7–13	8–17	11–23
% germination	†89.3	81.3	80.0	94.7	92.0	*84.3	88.0	90.7	89.3	78.7	69.3	65.7
% seedlings with anthracnose	84.0	74.7	57.3	62.7	29.3	4.3	13.3	1.3	0.0	0.0	0.0	0.0
% seedlings without anthracnose	5.3	6.7	22.7	32.0	62.7	80.0	74.7	89.3	89.3	78.7	69.3	65.7

Sunned seed No. 2

Date	1921						1922					
	6–25	7–2	7–9	7–16	8–10	10–29	2–25	4–15	6–5	7–13	8–17	11–23
% germination	†89.3	78.7	86.7	89.3	84.0	74.7	45.3	32.0	8.7	0.0	0.0	0.0
% seedlings with anthracnose	84.0	61.3	69.3	38.7	33.3	5.3	13.3	0.0	7.1	0.0	0.0	0.0
% seedlings without anthracnose	5.3	17.3	17.3	50.7	50.7	69.3	32.0	32.0	1.4	0.0	0.0	0.0

*70 seeds used.

† Data from a composite sample before sunning.

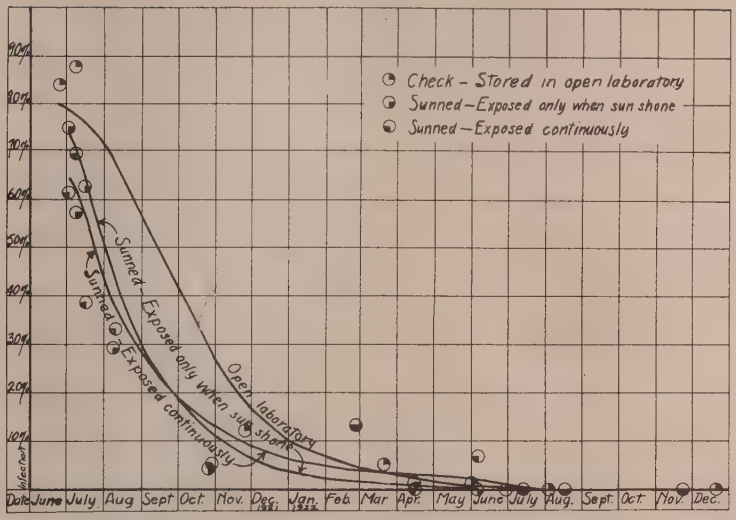

Fig. 11.—Curves showing the rate of decrease of the anthracnose fungus in sunned Toole cotton seed.

of the seed were spread out about an inch deep in wooden trays, which were then covered with wire netting to prevent rodent damage. These trays were placed on a light scaffolding outside a third story south window. With an occasional exception tray No. 1 was put out on bright days and taken in at night and during inclement weather. No. 2 was left out permanently except during some of the worst winter weather.

The results of this work are shown in table 15 and figure 11. The figures for the check sample stored in the laboratory are included for comparison. Unfortunately this test was begun, as the curves show, just about the time that the most rapid decrease in infection in the check sample was beginning. This circumstance, coupled with the lack of tests on the check for some months, makes it necessary to use caution in ascribing any beneficial results to this treatment. It will be noted however, that the decrease in infection was somewhat more rapid than under ordinary storage. On the other hand, it was

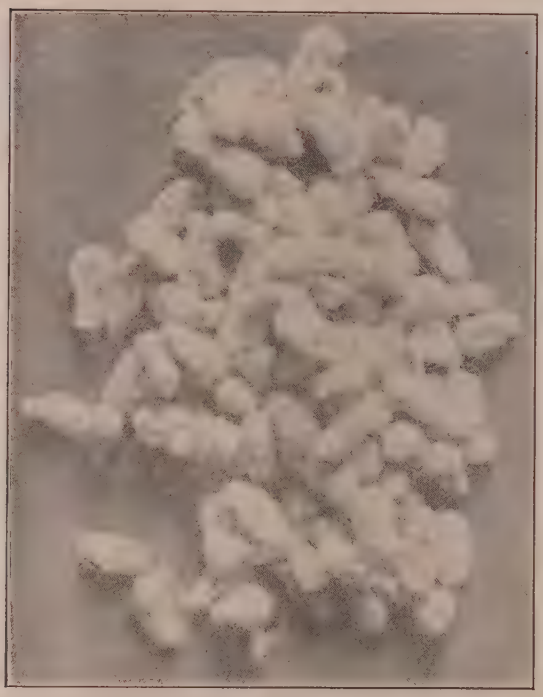

Fig 12.—Cotton seed before delinting.

not enough so to make the method of treatment of any practical value even if it could be accomplished with little or no trouble, which is very emphatically not the case. It will also be noted that lot No. 2 succumbed to the rigors of the winter and spring weather, and that by the close of the test the germination of No. 1, even, was impaired. While it is possible, of course, that an earlier treatment might have resulted more favorably for the treatment, the evidence secured indicates that there is not much virtue in sunning cotton seed as a means of destroying anthracnose infection.

APPLICATION

These studies do not reveal any quick method of eradicating cotton anthracnose from an infected sample of seed,

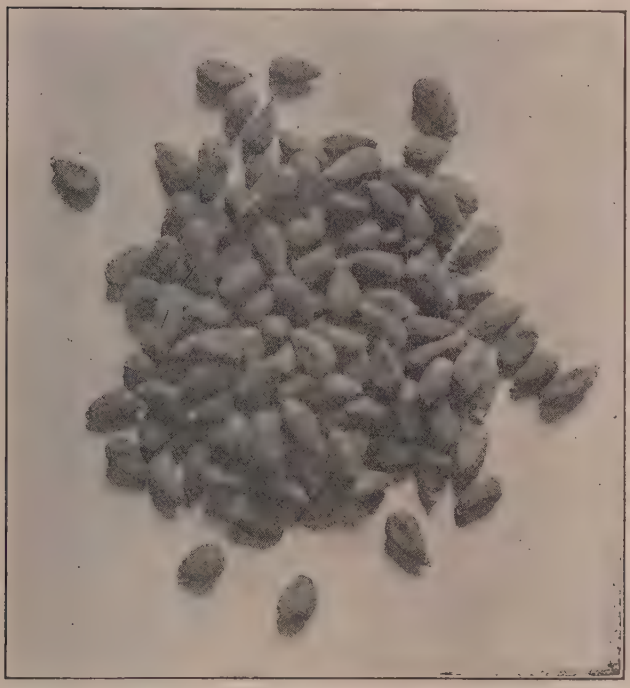

Fig 13.—Cotton seed delinted with strong sulphuric acid.

nor do they show any treatment or practical method of storage which will speed up greatly the death of the fungus during storage. They do show the value of delinting with strong sulphuric acid, however, as a method of removing a great part of the infection (Figs 12 and 13) ; and they verify the finding that infected seed become clean during storage. At the same time they indicate that storage till the third spring may be necessary in order to be sure that all infection is gone, especially if the seed have not been delinted and if storage is under farm conditions.

SUMMARY

1. Cotton seed infected with the anthracnose fungus, **Colletotrichum Gossypii,** becomes free of infection in storage.

Under laboratory conditions of storage this action proceeds very slowly until the seed become about a year old, when it becomes rapid. By the second spring after harvest the infection has practically disappeared.

2. No ordinary method of heating or drying the seed preliminary to storage seems to be of much value in destroying the infection, either by reducing the fungus directly or by rendering its subsequent life shorter during storage of the seed.

3. Delinting with sulphuric acid and sterilizing with mercuric chloride does help, however. Seed so treated have the initial infection cut down to a low figure, and that remaining in the seed seems to become eliminated a few months sooner than in untreated seed. It is probable that delinting alone would be sufficient for this purpose.

4. Storing seed in a very moist atmosphere produced the most rapid reduction in anthracnose infection of any means tried but the seed quickly became musty and failed to germinate.

5. Of the other methods of ordinary storage a location over a radiator seemed to be the most effective for control of the fungus and storage out-of-doors but under a roof least effective. Storage in the open laboratory and in a 30 degree incubator (temperature irregular) seemed neither so good as the one nor so poor as the other. It is to be remembered however, that the differences in the results from these methods of storage were small.

6. Storage in a very dry atmosphere, e. g., in a dessicator over calcium chloride, greatly prolongs the life of the fungus whatever the preceding treatment of the seed.

7. Storage in alternating very dry and very moist conditions seemed to induce a more rapid diminution of infection at first than is secured by ordinary laboratory storage; and there was little damage from mustiness; but the date of complete freedom from infection came little or no sooner.

8. Sunning the seed seemed to accelerate the death of the fungus somewhat but the results were so slow that the germination of the seed was seriously impaired before results of practical value were secured.

CONCLUSIONS

1. There is no quick means available for the elimination of anthracnose from cotton seed under ordinary farm conditions.

2. In the light of the results reported above the best treatment for an infected sample of seed is to delint with strong sulphuric acid, place in clean bags, and store two or three years in a dry building. A certain amount of artificial heat, as in a dwelling, is probably an advantage.

LITERATURE CITED

1. Atkinson, Geo. F. Anthracnose of cotton. Jour. Myc. **6**:173-178. **Pl. 17-18.** 1891.

2. Atkinson, Geo F. Some diseases of cotton. Alabama Agr. Exp. Sta. Bul. **41**: 65. **25 fig.** 1892.

3. Atkinson, Geo. F. Diseases of cotton. In U. S. Dept. Agr. Office Exp. Sta. Bul. **33**:293-299. 1896.

4. Balls, W. Lawrence. The cotton plant in Egypt. **xvi** -|- **202 p. 71 fig.** -|- **frontispiece.** MacMillian & Co., Ltd., London, 1919.

5. Barre, H. W. Cotton anthracnose investigation. Ann. Rpt. South Carolina Agr. Exp. Station **22**:89-118. 1909.

6. —Cotton anthracnose. Science n. s. **31**:68. 1910.

7. —Annual Report of the Botanist and Plant Pathologist Ann. Rpt. South Carolina Agr. Exp. Sta. **23**: 23-26. 1910.

8. —Plant disease survey of South Carolina. Ann Rpt. South Carolina Agr. Exp. Sta. **23**:29-39. 1910.

9. —Report of Botanist and Plant Pathologist. Ann. Rpt. South Carolina Agr. Exp. Sta. **24**: 19-23. 1911.

10. —Cotton anthracnose (Report of progress). Ann. Rpt. South Carolina Agr. Exp. Sta. **24**;23-43. 1911.

11. —Cotton anthracnose. South Carolina Agr. Exp. Sta. Bul. 164: **22 p. 7 pl.** 1912.

12. —Report of the Botanical Division. Ann. Rpt. South Carolina Agr. Exp. Sta. **25**:20-27. 1912.

13. —Report of the Botany Division. Ann. Rpt. South Carolina Agr. Exp. Sta. **26**:14-20. 1913.

14. —Report of the Botanist and Plant Pathologist. Ann. Rpt. South Carolina Agr. Exp. Sta. **27**:20-25. 1914.

15. —Report of the Botanist and Plant Pathologist. Ann. Rpt. South Carolina Agr. Exp. Sta. **28**:21-26. 1915.

16. —Report of the Botanist and Plant Pathologist. Ann. Rpt. South Carolina Agr. Exp. Sta. **29**:16-20. 1916.

17. —Report of the Division of Botany. Ann. Rpt. South Carolina Agr. Exp. Sta. **32**:29-34. 1919.

18. —Ann. Rpt. South Carolina Agr. Exp. Sta. (Plant diseases) **34**:17-22. 1921.

19. Barre, H. W. and W. B. Aull, Jr. The detection of anthracnose in cotton seed. Ann. Rpt. South Carolina Agr. Exp. Sta. **24**:43-49. 1911.

20. Bartlett, A. W., Diseases of cotton. Report Bot. Gardens (British Guiana) and their work, **1906-1907**:21. 1907.

21. Birmingham, W. A., and I. G. Hamilton. Diseases of the cotton plant. Agric. Gaz. New South Wales **34**:805-810, 877-886. 1923.

22. Butler, E. J. Fungi and disease in plants. **547 p. 206 fig.** Bibl. Thacker, Spink & Co.: Calcutta, 1918.

23. DeLoach, R. J. H. Some studies on the **Colletotrichum Gossypii** Georgia Agr. Exp. Sta. Bul. 85: **14 p. 8 fig.** 1909.

24. Duggar, J. F., and E. F. Cauthen. Experiments with cotton. Alabama Agr. Exp. Sta. Bul. 153:15-40. 1911.

25. Edgerton, C. W. The perfect stage of the cotton anthracnose. Mycologia **1**:114-120. 1909.

26. Edgerton, C. W. The rots of the cotton boll. Louisiana Agr. Exp. Bul. 137:**113 p. 13 pl.** 1912.

27. Jehle, R. A., R. Y. Winters, et al. Control of cotton anthracnose and improvement of cotton in North Carolina. Bul. North Carolina Department Agr. **41, No. 2**:14-28. 1920.

28. Lewton-Brain, L. Fungoid diseases of cotton. West Indian Bul. **4**:255-267. 1903.

29. Lewton-Brain, L. Further notes on pests attacking cotton in the West Indies. Fungiod pests. West Indian Bul. **4**:344-348. 1904.

30. Lewton-Brain, L. West Indian anthracnose of cotton. West Indian Bul. **5**:178-194. 1904.

31. Lipscomb, G. F., and G. L. Corley. A new treatment to destroy anthracnose. The Amer. Fertilizer **58, No. 6 (Mar.)**:32-34. **1 fig.** 1923.

32. Lipscomb, G. F., and G. L. Corley. On the vitality of cotton seed. Science n. s. **57**:741-742. 1923.

33. Penzer, N. M. Cotton in British West Africa. **53 p.** The Federation of British Industries. London, 1920.

34. Plant Disease Survey Staff. (Estimates of loss from plant diseases: cotton) Plant Dis. Bul. **2**:15. 1918. Plant Dis. Bul. Suppl. **6**:209 1919. **12**:329. 1920. **18**:334. 1921. **24**:507. 1922.

35. Shear, C. L., and Anna K. Wood. Ascogenous forms of Gloeosporium and Colletotrichum. Bot. Gaz. **43**:259-266. 1907.

36. South, F. W. Cotton diseases. West Indian Bul. **11**:75-77. 1911.

37. Southworth, E. A. (B. F. Galloway) Report of the Chief of the Division of Vegetable Pathology. In Report of the Sec. of Agr. **1890**:407-408. **1 pl.** 1890 (?).

38. Southworth, E. A. Anthracnose of cotton. Jour. Myc. **6**:100-105. **1 fig. 1 pl.** 1891.

39. Welles, Colin G. Two serious plant diseases new to the Philippines. Philippine Agriculturist **10**:253-254. 1921.